Declaration

UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Jan Vijg

#6
2/1/01

Serial No. 09/306,333 Art Unit: 1643

Filed: May 6, 1999 Examiner:

For: BRCA 1 and bMLHI Gene Primer Sequences And Method For Testing

DECLARATION

R. David Rines declares as follows:

1. For several years and at all times pertinent herein I have served on the Harvard Medical School-Beth Israel Research team of Dr. Vijg in developing the application of the Vijg gene-scanning techniques described in the Vijg references cited by the Examiner, and I am fully familiar with the procedures and work done by and with Dr. Vijg; and, more particularly, the advanced work on BRCA1and hMLH1 gene primer sequences and scanning disclosed in the above-entitled application. In fact, I personally was involved in developing the BRCA1 primer sequences listed in Tables 4 and 2 of the above application.

2. I have noted the suggestions of the Examiner in the Office action of May 16, 2000 in the above-entitled application that the providing of a showing of improved and unexpected results with the primer pair sequences of Tables 1 through 4 of the application over the general teachings by Dr. Vijg's earlier publications, particularly for other genes than the BRCA1 and hMLH1, may serve to overcome a holding of possible obviousness of the results of the above application.

3. Adherence structures to the prior teachings of the application of the general Vijg technique to RBI genes is not adequate for obtaining satisfactory results with either the BRCA1 or the MLH1 gene for the following reasons.

4. With regard to the RBI gene, complete exon coverage in a single fragment was obtainable with the Vijg technique. As is discussed in the above application, this is not possible with the BRCA1 or the MLH1 genes which require splitting the fragments. In the case of the BRCA1, furthermore, it was discovered that even splitting in half, or a few splittings, was not adequate. Indeed, as shown with exon 11, a modified concept of splitting a large number of times--(in this case 16 times--11.1 through 11.16) was found to be required--a radical departure from the former teachings of the Vijg technique, in

connection with the earlier RB1 gene or the P53. More than that, clamps of variable sequence and links were found to be necessary to induce a stable melting domain--specifically the pairs of different clamping units shown for exons 11.1, 11.2, 11.5, etc. in Table 4 of the specification of the above application. These were found to be required to obtain the rather remarkable resolution, clarity and reliability shown in the photo of Fig. 1B of the BRCA1 and in Fig. 1A for the MLH1. In connection with the former, exon 11 is shown split into 16 divisions which were certainly a radical departure from the former teachings of the Vijg technique, in connection with the earlier RB1 gene.

5. Further in connection with the MLH1gene, and again, unlike the single clamp pair primer pairs used with the scanning of the RB1, variable sequence clamps were found to be required to induce the desired stable melting domain; specifically fragment 12.2 (clamps 8 and 40) and fragment 14 (clamps 45 and 5).

The repeatable reliability and improved resolution and clarity results before-discussed as attained with these departures from the prior Vijg procedures represented, in our view, an important step forward in the detection of mutations and normalcy of the specific genes addressed in the above application--results which, to our knowledge, certainly were not obtained prior to the present application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Sec. 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: October 25, 2000

R. David Rines